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Citrus blast and black pit, which were formerly considered to be due to two different species of bacteria, are now known to be simply different manifestations due to the same organism, *Pseudomonas citriputeale* C. O. Smith.[1, 2] Citrus blast is the name which was given to an effect of this bacterium on the leaves and twigs, and black pit the name given to a different effect upon the fruit.

The first of these effects to be reported was black pit, described by C. O. Smith in 1913[2] as a brown or black sunken spot occurring on lemon fruits grown in southern California. His attention had been first called to the disease in 1909. The causal organism was named by him *Pseudomonas citriputeale.*[2] Citrus blast was first described as a new disease of leaves and twigs by Coit, 1916[3] Hodgson reports having observed it as a new disease as early as 1912.[4] The organism was found on citrus blast lesions and named in 1917 *Bacterium citrarefaciens* by H. A. Lee.[5]

It is the purpose of this bulletin to report the results of investigations begun in 1919[6] to determine more definitely the nature of the disease, the conditions influencing its development, and the means to be employed for its prevention and treatment.

* Paper No. 102, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

DISTRIBUTION

The disease was first thought to be confined principally to the citrus orchards of northern California, especially those of Butte County. It was afterwards found to be fairly well distributed throughout northern California, and a few cases were found in Tulare County. The phase of the disease on the fruit known as black pit, which was considered at first to be a different one, had been found by C. O. Smith in various localities in southern California, but the typical lesions on the twigs and leaves were not definitely observed in southern California until the past year.

It now appears evident that the organism responsible for these two manifestations is rather generally distributed throughout the sections in which citrus is grown, and that the manifestations on the leaves and twigs known as blast are especially dependent for their occurrence upon the right combination of weather conditions with suitable mechanical injuries. In the spring of 1922, one of the groves highest up on the foothills near Pasadena showed a considerable outbreak of blast upon the twigs and leaves, and of black pit upon the fruit. The rainfall in this orchard between January 1 and May 1 was about thirty inches, and this, together with the unusual number of cool, cloudy days, produced conditions which were quite similar to those which occur on the foothills near Oroville, where the citrus blast is usually severe. It therefore seems likely that both citrus blast and black pit may appear whenever suitable weather conditions occur.

SYMPTOMS AND DEVELOPMENT

As stated above, this disease occurs in two forms, one on the leaves and twigs and the other on the fruit. Its activity is confined to a limited period, usually extending over six to eight weeks in the winter and spring. Oranges and grapefruit are probably the most susceptible to the injury upon the leaves and twigs, while lemons are the most susceptible so far as the fruit is concerned.

The most striking characteristic of the blast manifestation is an area shading from brown to black, which usually starts in a tear or break on the wing of the petiole (see pl. 1, fig. A) and extends rapidly in both directions to the base of the leaf blade and to the twig surrounding the base of the petiole. On January 22, 1921, at Oroville, out of thirty-eight lesions taken at random thirty had started on the wing of the petiole at some place between the leaf blade and twig; seven had started at the base of the leaf blade; and only one had originated on the twig near the base of the petiole. During cool, damp weather these areas enlarge rapidly, but when the dry and warmer weather comes on the progress of the lesions is checked. The following features are to be noted: there is a definite line between the dead and live tissue; new callus tissue forms under the affected areas; the surface of the affected bark becomes dry, and the initial black color changes to a reddish brown, forming scabs (pl. 1, figs. B and C). If the disease progresses very rapidly the leaves wither and die while still attached to the tree (pl. 2), but if its progress is slow or if it occurs in a somewhat warmer temperature, an abscission layer is formed and the leaves are shed. In the latter case, it seldom progresses farther than the petiole of the leaf, and few lesions will be formed on the branches. In very severe cases the disease may progress so rapidly as to girdle the twigs and cause many of them to wither and die; but more frequently the lesions are checked before girdling takes place. Microscopic examinations have shown that the bacteria are most abundant in the layers not far from the cambium. They appear to attack the parenchyma tissue principally, and do not kill the woody tissue except in extreme cases.

A corky brown scorching, or staining, of the surface layers of leaves often accompanies the blast. This sometimes spreads over a portion of the leaf blade beyond the edge of the lesion (pl. 3, figs. A and B). Its occurrence under the same conditions as those which produce blast has indicated that the citrus blast organism may have some relation to this manifestation. It differs from the ordinary form of blast in that only the very outermost layers of cells are affected, while the affection does not cause the death of the tissue beneath. Some experiments have shown that a reddish brown scorching similar to that observed can be produced by rubbing the under surface of the leaves with a blunt instrument and painting on a suspension of the

organism. The leaves which were rubbed without painting with this organism simply took on a grayish discoloration without any reddish brown color.

As black pit the disease manifests itself on the fruit, especially in the case of lemons, as sunken black spots (pl. 5). As these develop they change from a light brown to dark brown, and then to black. The inner white part of the peel is also affected. There is a collapse of the tissue, which finally becomes light brown tinging to reddish brown in color. The size of these spots is dependent upon the conditions of moisture and temperature under which they develop. On lemons they are mostly from one-fourth to one-half an inch in diameter, but many attain a size of three-fourths of an inch. If the spots occur close together, they may be smaller. These black pit spots may also occur on oranges and grapefruit (pl. 4), but are usually not as common nor as deep as upon the lemon fruits. As in the case of citrus blast, the black pit spots are usually dependent upon injuries.

IDENTITY OF CITRUS BLAST AND BLACK PIT ORGANISMS

While work was being done with cultures of the citrus blast organism obtained from northern California in the spring of 1920, isolations of bacteria also were made from some black pit spots on lemons obtained from Alta Loma in southern California. The similarity in the appearance of the cultures from the two sources was noticed, and parallel inoculations were made on lemon fruits by puncturing them on one side with the organism from citrus blast lesions and on the opposite side with the organism obtained from black pit. Black pit spots, identical in their manner of development, were obtained in both cases. These results led to the suspicion that we were dealing with the same species of organism and not with two different species, as had formerly been supposed. Experiments of this kind were repeated several times with the same results (pl. 5). It was also found that typical blast lesions could be produced on short shoots of citrus kept in moist chambers in the laboratory. Parallel inoculations with these organisms from the two different sources showed them to have the same effect in producing blast lesions on these cuttings. Some of these experiments were repeated at Berkeley by A. F. Camp, both in

the laboratory on fruit and in the greenhouse on growing plants, with the same results. Later, at Oroville, the organisms from two different sources were again tested by a number of inoculations, both in moist chambers and in the orchard. The results here again indicated the identity of the organisms from the two sources. Since that time the organism has been isolated repeatedly from twig lesions and upon being used for inoculation has been found to produce black pit. Likewise, it has been isolated repeatedly from black pit lesions and caused to produce the blast lesion on twigs (pls. 5 and 6).

Culture and Staining Tests.—The organisms from the two sources (blast lesions of orange twigs and black pit lesions of lemon fruits) have been carefully compared in parallel cultures and in staining tests. Most of the tests reported both by C. O. Smith and H. A. Lee were repeated and others added. In all these tests the cultures from the two sources were alike in their reactions. No differences that could possibly separate them as species were detected. The organisms from the two sources were also compared on a few of the standard media with one of C. O. Smith's original cultures, which have been kept alive by transfers since 1913. The results were identical. We have found the characteristics of the organisms to agree with Smith's description of *Pseudomonas citriputeale,* with the exception of the number of flagella, which is usually few to many, instead of one. Lee found one to four, but there are usually many more than four.

The results of our tests differ from Lee's technical description of *Bacterium citrarefaciens* in at least two particulars, and from his general description in several others. We found, as did Smith, no coagulation in milk, and the green color mentioned by Lee as a prominent characteristic was absent in almost all media. Only rarely, and only on standard agar containing considerable glucose, was there even a slight suggestion of green, and then only with certain strains of the organism. It is possible that Lee was dealing with a strain of the organism with an exceptional tendency toward the production of a green pigment. In other respects our results differed from Lee's in that no growth was obtained either in Uschinsky solution or in the closed arm of fermentation tubes of maltose. Our results agreed with Lee's in that growth was obtained in the closed arm of tubes with dextrose and saccharose, and none in the closed arms with lactose. In most other respects our results were also in agreement with Lee's general description.

The organism from the two sources produced practically the same effect on the H-ion concentration of various culture media. In various sugar solutions (two per cent) in fermentation tubes with an initial pH of about 7.4, the comparative results are as shown in table 1.

TABLE 1

Source	pH in 34 days in			
	Saccharose	Dextrose	Maltose	Lactose
Blast	4.4	6.8	6.8	7.4
Black pit	4.5	6.8	6.8	7.6

Inoculation Tests.—Inoculations were made May 17, 1920 on lemon fruits, to compare the effects of bacteria isolated from citrus blast lesions on orange twigs from northern California with that of bacteria isolated from black pit lesions on lemon fruits from southern California. Four lemons (two light green and two yellow) were placed in each of seven temperature chambers after being inoculated. One side of each fruit was inoculated by puncturing with a steel needle in four different places in the case of the northern organism, and the other side inoculated in a similar way in the case of the southern. The effects produced by the organisms from the two different sources were quite similar, as shown in table 2.

TABLE 2

DIAMETER OF BLACK PIT SPOTS ON LEMON FRUITS THREE DAYS AFTER INOCULATION
(Average in mm. of six to eight punctures on each of two fruits)

Source of cultures	Kind of fruit	14° C. 57° F.	17° C. 64.5° F.	20° C 67° F.	24° C. 75.3° F.	27° C. 80.6° F.	30° C. 86° F.	33° C. 91.5° F.
Blast lesion, Oroville	Light green	7.0	5.4	6.2	3.7	3.2	1.0	0
	Ripe	5.2	7.2	7.9	4.9	6.0	1.6	0
Black pit lesion, Alta Loma	Light green	6.5	5.4	5.7	3.3	4.8	1.8	0
	Ripe	5.4	7.1	4.7	3.7	5.6	1.6	0

No lesions developed at 33° C. (91.5° F.). At 30° C. (86° F.) there appeared to be a slight temporary effect, but no development of lesions. At 27°, 24°, 20°, 17°, and 14° C. (80.6°, 75.3°, 64.5°, and 57° F.) black pit lesions developed. In three days the largest spots in diameter on green fruit were produced at 14° C. (57° F.), but those on the fruits kept at 17° and 20° C. (64.5° and 67° F.) were

nearly as large and more sunken. The largest spots developed on ripe fruit at temperatures of 17° and 20° C. (64.5° and 67° F.). A fairly cool temperature, such as 14 to 20° C. (57 to 67° F.) is most favorable for the development of lesions, but lesions may develop at constant temperatures as high as 27° C. (80.6° F.). In general, the organisms from the two sources acted alike in producing black pit.

A comparison of the size of colonies of the bacteria from blast and black pit lesions in different temperatures after three days is shown in table 3. The inoculations were made by bringing a one-millimeter loop from a bouillon culture in contact with the surface of solidified soluble-starch agar.

TABLE 3

SIZE OF COLONIES (MM. IN DIAMETER) IN THREE DAYS, ON SOLUBLE STARCH AGAR PLUS ONE-HALF PER CENT GLUCOSE

(Average of about six colonies in three petri dishes)

Source of culture	Temperature						
	13° C. 55.4° F.	17° C. 64.5° F.	20.5° C. 69° F.	24.5° C. 76° F.	27° C. 80.6° F.	30° C. 86° F.	34° C. 93.2° F
Blast lesion, Oroville	4.3	6.0	8.0	4.8	3.4	3.0	0
Black-pit lesion, Alta Loma	3.5	6.5	7.6	5.8	3.1	3.0	0

There appeared to be no significant difference between the cultures from the two different sources in their reaction to temperature. The largest colonies were formed at 20.5° C. (69° F.). No growth took place at 34° C. (93.2° F.).

EXPERIMENTS IN ISOLATING THE ORGANISM

The blast organism has been isolated not only from the leaf and stem lesions, but also from black pit spots on lemon, grapefruit, and orange fruits. It was also readily found in drippings both from infected and sound leaves during and following rains. The organism was isolated readily from active lesions, but not so easily from older lesions that had ceased enlarging.

Out of a considerable number of tests from healed-over lesions a year or more old, a few yielded organisms capable of producing black pit on lemon fruits. The results appear to point to a strong probability that the organism is carried over in some of these lesions

at least, even after the lesions are healed over and appear to be inactive. Lee reports having isolated the organisms from inactive lesions after the scabs had formed.

Certain cultural and inoculation tests, made to determine whether the organism was common in the soil and air in an infected orchard, failed to give any positive results. These tests were not extensive, but they indicated that the blast organism was not abundant in the particular samples tested, since the organism could be readily detected in other samples of soil, to which the blast organism had been purposely added.

INOCULATIONS ON DIFFERENT SPECIES OF CITRUS AT BERKELEY

During September and October, 1920, a number of sets of inoculations comprising several leaves each were made at Berkeley on trees in a moist atmosphere in an outdoor inoculation chamber devised by Camp.[7] The surface of the leaves in this chamber was kept almost continually moist. Inoculations were made by puncturing with a needle and by hypodermic injection into leaves of sweet orange, sour orange, tangerine, and Eureka lemon. The temperatures in the chamber ranged from about 10 to 17° C. (50 to 62° F.). Positive results in producing blast lesions on leaves were obtained in the case of all these varieties, definite evidences of development starting usually in from about three to six days.

On the sweet orange the evidences of the development of the disease were noted in from three to six days. On growing and recently matured leaves they developed in from three to four days. One inoculation, which had begun within four days from a needle scratch, spread along the petiole and seven millimeters along the stem in twenty-one days. On older leaves a lesion which had started within six days had spread down the petiole and three millimeters along the stem in twenty-two days.

Recently matured sour orange leaves showed positive beginning of lesions in from three to four days, and more mature leaves in from seven to eight days. No striking difference in resistance to spread of lesions was noted between the sweet and sour orange.

Dancy tangerine leaves, recently matured, showed positive lesions in from three to six days in different experiments, and displayed the same characteristics as in the case of the sour or sweet orange leaves.

On rapidly growing Eureka lemon leaves, the evidences of development were seen in from three to eleven days. The spread of the lesions stopped sooner than on the orange leaves, indicating a greater degree of resistance to invasion of the organism. Leaves of all these varieties which were atomized but not punctured or injured failed to produce blast.

In April, 1921, hypodermic injections in the case of matured orange leaves started positive lesions in six days, but in the case of young leaves only slight or negative effects were produced.

DOES THE CITRUS BLAST ORGANISM ATTACK OTHER HOSTS?

While observations on citrus blast were being made in the Oroville section, lesions which resembled those on citrus trees were discovered on the leaves and stems of the interior live oak, *Quercus wislezenii.* These oaks were in the vicinity of orchards affected with citrus blast. Trees of the same species of oak growing far back in the foothills and mountains were examined, and were also found to contain these lesions. The lesions have not been found in great numbers on this oak, nor do they appear to damage the oak as severely as they do the citrus. Similar lesions were also found on the same species of oaks growing on the foothills in Tulare County. A number of isolation tests were made, but no cultures of the blast organism were obtained from these naturally occurring lesions. Active, fresh lesions on the oak have not been tested.

That lesions of this nature, however, may be produced on oaks by the citrus blast organism has been shown by the following experiments.

Small oak trees, *Quercus wislezenii,* were transferred to the lathhouse of the Citrus Experiment Station and the following year inoculations with the citrus blast organism were made in punctures on the very new growth. Petioles of young leaves and growing stems of three plants were punctured with a hypodermic needle containing a suspension of the organism, and the plants were covered with bell jars. Lesions were produced similar to those which had been observed

as occurring on this species in the field. The organism was again isolated from these lesions, after they had developed for about eight days. Twigs of this oak, growing rapidly when cut, were also inoculated in moist chambers held at different temperatures. The best development of lesions was at 22.5° C. (73° F.) and occurred in five days. Definite lesions developed at 13° C. (55.4° F.) in nine days.

Lesions had previously been produced on the coast live oak, *Quercus agrifolia,* at Berkeley. Two leaves of this oak were punctured on April 5, 1920. Positive lesions had extended twelve millimeters along midrib and petiole and ten millimeters along the stem in seven days. The organism was then isolated from this lesion and produced black pit on lemon fruits.

In June, 1920, bits of tissue from lesions found on oaks, *Quercus wislezenii,* at Oroville were inserted in the rind of lemon fruits with the result that typical black pit lesions were formed.

These experiments and observations afford strong evidence that certain species of oaks common in California are attacked by the citrus blast organism. Lesions have been produced on the oaks by inoculation with the organism from the citrus, and cultures of the same organism have been recovered from these oak lesions. It remains, however, to isolate the organism from the naturally occurring lesions on the oaks, and to produce with it the blast upon the citrus. Although the later part of the proof has not as yet been obtained, the originators of the theory believe it is only a question of getting the oak lesions at the time of their most active development.

From March 23 to April 2, 1921, the following attempts were made at Berkeley to produce lesions on deciduous fruit trees with cultures of the blast bacterium.

(1) Cherry: Flowers and leaves atomized with suspension of the organism, terminal buds and leaves injected with hypodermic needle with suspension, young leaves and shoots punctured.

(2) Apricot: Young leaves atomized, buds and growing shoots injected with hypodermic needle.

(3) Pear: Young growing leaves atomized, buds injected.

(4) Almond: Young growing leaves atomized, buds and growing shoots injected.

All these tests failed. Positive lesions were, however, produced on mature sweet orange leaves under the same conditions.

RELATION OF CITRUS BLAST TO ENVIRONMENTAL CONDITIONS

Relation to Moisture

Observations have indicated that the severity of citrus blast injury was dependent to a considerable extent upon the moisture conditions during the early part of each year, usually from January to March. The disease was quite severe during some years and mild or of small importance in other years. It also seemed evident from observation that when the leaves were covered almost continuously for several days with a film of water during cool rains, the disease developed rapidly, while its development was slower when the weather was such as to allow the foliage to become dry. In accordance with Hodgson's observations from 1912 to 1918, and ours of more recent date, the past eleven years have been divided into two groups, one in which the blast was said to be severe and the other in which it was considered at Oroville to be mild. The following table shows the rainfall, number of days of rain and of totally cloudy days for the period between January 1 and March 31 for each of these years.

TABLE 4

Rainfall, Number of Rainy and Cloudy Days for January, February and March, at Oroville, in Relation to Citrus Blast

Citrus blast severe (years)	Rainfall	Days of rain	Cloudy days
1915	23.52	36	60
1916	20.28	44	55
1919	14.28	37	51
1921	11.15	30	21
1922	12.77	37	35
Citrus blast mild (years)			
1912	6.26	23	48
1913	9.16	20	27
1914	15.80	24	39
1917	10.01	19	27
1918	12.93	26	49
1920	7.26	23	38

TABLE 5

RAINFALL, NUMBER OF RAINY AND CLOUDY DAYS FOR JANUARY, FEBRUARY AND MARCH, AT RIVERSIDE, WHERE LITTLE OR NO CITRUS BLAST OCCURRED, DURING THE SAME PERIOD AS SHOWN IN TABLE 4

Years	Rainfall	Days of rain	Cloudy days
1915	8.6	21	18
1916	12.47	28	10
1919	4.60	18	13
1921	5.03	16	14
1922	8.58	22	14
1912	.75	15	12
1913	4.19	5	4
1914	10.01	20	20
1917	4.25	17	13
1918	8.32	18	12
1920	7.74	23	21

It is seen that the records for the number of rainy days in the severe group of years at Oroville are higher than those in the mild group of years. This feature of the weather is probably the most important in its influence on citrus blast. The number of totally cloudy days in general was higher in the severe years than in the mild years and this is also true of the rainfall. In 1921, one of the severe years, when the rainfall was comparatively light while the number of rainy days was fairly large, the blast appeared early because of heavy December rains which are not included in this record. In 1914, one of the mild years, the rainfall for the period was fairly heavy, but the number of rainy days remained few and was small in February and March. A study of the weather data for the same period at Riverside shows that in only one year, 1916, of the eleven recorded, did the number of rainy days approach even the lowest figure for the severe years at Oroville, and in most years it was below the average for the mild years at Oroville. The highest number of cloudy days at Riverside for the period covering the eleven years, was considerably below the lowest at Oroville. This is probably one of the main factors in the situation, at least it may account for the slight occurrence of citrus blast in sections like Riverside.

That the number of rainy days may be an important factor is further borne out by the fact that this past year citrus blast occurred to a considerable degree in an orchard on the foothills below Mt.

Wilson near Pasadena, where the rainfall from January to May was about thirty inches, accompanied by much fog, severe winds, and cool weather resembling that of normal years at Oroville. The strong winds producing the necessary injuries near Pasadena were from the northeast, and it was also on the northeast side that the majority of the citrus blast lesions were found.

Relation to Injuries

After a careful study of the development of the lesions and of inoculation tests, it has been determined that infection leading to development of blast or black pit usually takes place in slight rifts, tears, scratches, thorn pricks, scars, rubbings, or other injuries and does so most readily if these injuries are fresh and kept moist by rains or fogs. The data in tables 6 and 7 show the percentage of

TABLE 6

Percentage of Injured Leaves at Oroville on Ten Six-Year-Old Trees with Long, Vigorous Shoots

Date	Number of twigs	Total number of leaves counted	Number of leaves injured	Percentage injured
February 8, 1921				
South half	47	751	468	62
North half	52	764	420	55
February 15, 1921				
South half	44	654	390	60
North half	39	485	242	50

TABLE 7

Date	Leaves injured			Leaves not apparently injured		
	Total number	Number with blast	Percentage of blast	Total number	Number with blast	Percentage of blast
February 8, 1921						
South half	468	317	67	283	0	0
North half	420	165	37	344	0	0
February 16, 1921						
South half	390	266	68	265	2	0.8
North half	242	124	50	248	5	2.0

injured leaves on similar twigs taken to represent the north and south half of the tree and also the corresponding percentage of blast.

The percentage of leaves injured, as shown in table 5, was somewhat less on the north than on the south side of the trees. These trees were of an open type of growth. More compact trees would probably have shown very striking differences in the two sides. The blast lesions which had developed from injuries were also considerably less abundant on the north side, away from the prevailing winds, than on the south side. Calculation shows in the one case that 41.5 per cent of the leaves on the south were diseased, as compared with 20.3 per cent on the north side; and in the other set, 40.8 per cent of leaves on the south side as compared with 25 per cent on the north side.

An analysis of the kind of injuries on the leaves and their relation to citrus blast was also made on February 15, 1921. The data are shown in table 7.

TABLE 8

KIND OF INJURIES AS RELATED TO CITRUS BLAST AT OROVILLE, FEBRUARY 15, 1921

Kind of Injury	Leaves with blast			Leaves without blast		
	South	North	Total	South	North	Total
Breaks: mostly a rift in one wing of the petiole	221	111	332	26	26	52
Punctured or torn places, mostly in the leaf blade	15	6	21	2	3	5
Rubbed places	6	2	8	3	0	3
Injuries by Fuller's rose beetle	24	5	29	92	84	176
No apparent injuries	2	5	7	262	243	505
Grand total		397			741	

It is here seen that the main portion of the citrus blast lesions developed from a certain type of injury in the wing of the petiole such as is shown in plate 2, figure A. The disease may also develop in other punctured or torn places in the leaf blade, in rubbed places, and in injuries made previously by Fuller's rose beetle. In this last case, however, the proportion of lesions to the whole number of such injuries was rather small, probably because of the fact that they were made before the active period of the blast began. A few lesions were found where no apparent injury could be detected with a hand

lens, although it was not ascertained with absolute certainty that none of the cells had not been previously injured.

In contrast to these records, which were taken from trees exposed to the wind, a total of 712 leaves was counted on similar growth on the south side of trees protected from south wind by large olive trees. Of these, only thirty-four, or about 8 per cent, were injured. Lesions had developed in thirty-one instances, or 23 per cent, of these injuries.

Relation to Temperature

Temperature, especially within certain limits, also has an important influence on development of citrus blast, as shown in tables 2 and 3. In an orchard under natural conditions it is especially difficult to estimate the influence of temperature apart from humidity, since the latter usually varies with the former. The temperature, of course, influences the host as well as the parasite, which makes an analysis of the temperature influence difficult.

In an orchard in the Wyandotte section where blast was developed actively in 1921, temperature records showed the following average daily maximum and minimum temperatures for January, February, and twenty-four days in March.

TABLE 9

Average Temperatures in Orchard near Oroville, while Citrus Blast Was Active

	Maximum (F.)	Minimum (F.)
January	50°	37°
February	64°	45°
March 1-24	77°	52°

The humidity record, which was kept during February, showed an average maximum of 96 and an average minimum of 53.

It has been observed in Butte County that in the spring, soon after the weather becomes warmer and dryer the active development of lesions no longer takes place. It is probable that a change in the host may be an important factor in this instance, as it is in so many plant diseases; but temperature is also thought to be of importance. In

addition to the influence of the temperature during development of the disease, that of previous chilling of the host appears to be a factor in some cases, although it is not a necessary condition for disease development.

An attempt has been made to obtain some light on the temperature relations by investigating the development of the organism and the lesions in artificial inoculations in constant-temperature chambers. Some experiments in the transfer of inoculated tissue from one temperature to another have been also carried on. Inoculations of cut twigs from recent growth of sour and sweet orange were made on February 24, 1920, and put into moist chambers in a supposedly saturated atmosphere. Needle punctures containing blast bacteria were made on these twigs near the base of petioles, and check punctures without inoculum were made on other twigs. In two days there was definite indication of development of the disease at 13, 16.5, 19, 22.5, and 26° C. (55.4, 62, 66.5, 73, and 78.8° F.). In four days the largest lesions, which were two to four millimeters in their longest diameters, were produced, the temperature being 19° C. (66.5° F.). Slight discoloration at punctures and gum exudation was noted at 29 and 33° (84.2 and 91.5° F.). The checks at this temperature showed no gum. No gum or other indication of disease was noted at 40° C. (104° F.). At this temperature the leaves turned brown in four days, as if they had been cooked. The sour orange shoot appeared to resist the heat longer than the sweet orange shoot. The moist chambers containing the twigs were then taken out of their respective temperature chambers and placed together in the laboratory at about 16 to 20° C. (60.8 to 68° F.). Within six days the greatest lesions appeared to have developed on twigs which had previously been kept at 13° C. (55.4° F.). On these the lesions were large and definite, being five to seven millimeters in their longest diameter. The sour orange tissue appeared to be slightly more resistant to the development of lesions than the sweet orange.

Another set of somewhat older cuttings of sweet orange were inoculated on March 29, 1920, and developed their largest lesions in five days at 22.5° C. (73° F.); while those kept at 13 and 19° C. (55.4 and 66.5° F.) were also well started. Gum exudation took place at 26° C. (78.8° F.). In nine days the lesions at 13° C. (55.4° F.) were very marked. Cuttings of recently grown twigs of

Quercus wislezenii inoculated at the same time also developed definite lesions at 22.5° C. (73° F.) in five days and at 13° C. (55.4° F.) in nine days.

On April 27, 1922, inoculations were made on yellow lemon fruits by transferring a two-millimeter loop full of bouillon culture and puncturing the surface of the rind through this drop. The fruits were then placed in moist chambers at different temperatures. The average sizes of spots developing in three and in five days, respectively, are shown in table 10.

TABLE 10

SIZE OF BLACK PIT LESIONS DEVELOPING IN FIVE DAYS AT DIFFERENT TEMPERATURES

(Average of about twenty-four punctures on four yellow lemon fruits in each temperature)

	Temperature						
	8.5 47	12.5 54.5	17 62.6	20.5 69	25 77	28.5 83.4	33.5° C. 92.5° F.
Size of lesions in mm. after 3 days	2.0	3.5	6.6	6.6	6.3	3.3	3.1
Size of lesions in mm. after 5 days	2.33	5.1	8.0	6.6	6.3	3.3	3.1

The spots averaged about equal as to diameter on the third day, the temperature being 17, 20.5, and 25° C. (62.6, 69, and 77° F.). They were more sunken at the highest temperature. In five days the spots developing at 17° C. (62.6° F.) were decidedly larger than at any other temperature. Those developing at 20.5° C. (69° F.) or above did not increase after the third day. Those at 33.5° C. (92.5° F.) were not typical, deep-seated spots, and probably developed rapidly previous to the death of the organism. Those at 28.5° C. (83.4° F.) did not enlarge after the second day.

On May 10, 1922, an experiment with recently grown twigs from navel orange trees was started. These were inoculated by cutting through the wing of the petiole with a scalpel carrying the organism and by puncturing, and were kept in a saturated atmosphere, as in previous experiments. In five and seven days the most pronounced lesions were found to have developed at 17 and 20.5° C. (62.6 and 69° F.). Small lesions with gumming and abundant callus were pro-

duced at 28.5 and 33.5° C. (83.4 and 92.5° F.). Lesions were quite definite at 12.5° C. (54.5° F.). Slight but definite development also occurred within this time at 7.5° C. (47° F.). At the end of seven days some of the twigs which had been at 7.5° C. (47° F.) were placed in a temperature of 20.5° C. (69° F.), and some of those at 20.5° C. in a temperature of 7.5° C. In six days more those transferred from 7.5° C. to 20.5° C. had increased rapidly, forming large individual lesions about equal in size to those which had remained for the entire time at 12.5° C. Those transferred from 20.5 C. to 7.5° C. developed very little during these six days, while the lesions developed at 12.5° C. were the most distinct and measured about five by two millimeters. These experiments indicate that temperatures of about 20° C. (68° F.) or below are most conducive to the development of citrus blast lesions, and that lesions may develop well at a constant temperature of 12.5° C., while they develop slowly at temperatures as low as 8.5° C.

There is also a suggestion that chilling followed by higher temperatures may render the tissues more susceptible to the invasion of the organism. This is in keeping with the observation that citrus blast is severe after periods of low temperatures not sufficient to freeze the tissue. The development of the citrus blast lesions prevalent in southern California this year (1922) may have been promoted by the low temperatures which prevailed from January 18th to 22nd, and were followed by a large number of rainy days and moderately cool weather and winds in March and April.

Inoculations made by injecting bacteria with a hypodermic needle into the leaves of sour orange one-year-old seedlings, growing in pots, were made at different temperatures on May 9, 1922. The trees had just begun new growth, but the injections were made in the older leaves. One plant with three leaves injected was placed at each of the following temperatures and given artificial light for nine hours each day: 8, 12.5, 17, 21, 24, 28.5, and 33° C. (47, 54.5, 62.6, 69, 77, 83.4, and 92.5° F.). Checks were injected with distilled water and kept at 8 and 21° C., but produced no spots. Dead spots were produced by the inoculations at all these temperatures. There was no enlargement after the second day in the case of leaves at 33° C. (91.5° F.), and none after the third day in the case of those at 28, 24, or 21° C. (83.4, 75.2, or 70° F.). The lesions on those at 17, 12.5,

and 8° C. (62.6, 54.5, and 46.6° F.) continued to increase in size up to the eighth day, when the plants were taken out and all placed at a temperature of 18 to 20° C. (64.5 to 68° F.). In the next six days the lesions on the one which had been at 12.5° C. (54.5° F.) increased rapidly and caused more injury than any of the others. Those on the one which had been at 17 and 8° C. (62.6 and 46.6° F.) also increased somewhat. Those on the one which had been at 21° C. (70° F.) or above failed to increase after removal from the chambers, and the lesions appeared the same size as they were at the end of the third day. The spots on the leaves at the higher temperatures varied from dark to reddish brown, and those on the leaves at the lower temperatures from dark brown to black in color.

Culture tests on glucose potato agar, made by stabbing the surface of the solidified medium in petri dishes with the end of a needle, showed the largest colonies at 24° C. (75.2° F.) in one, two, and three days. In five days the colonies were almost the same size as they were at 17, 20, 27, and 29° C. (62.5, 68, 80.5, and 84.2° F.), but very small and they were probably dead at 33° C. (91.5° F.). A fair growth took place at 10 and 14° C. (50 and 57° F.). The cultures from blast lesions in the north reacted in a similar manner to the culture from black pit lesions of the south.

CONTROL

Experiments on Prevention

Spraying experiments have been carried on during three consecutive years, and the results each year have indicated that Bordeaux mixture, if applied as early as the first of November, will prevent a considerable part of the injury from this disease. It is of small advantage if applied some weeks later. None of the other mixtures tried, with the exception of mercuric cyanid (one part to 500 parts of water), were nearly as effective.

On October 28, 1919, a block of sixteen navel orange trees at Oroville were sprayed with Bordeaux mixture (5–5–50) and another block of twelve trees with mercuric cyanid (1–500). These trees were five years of age, and were surrounded by similar trees of the same age, but unsprayed. They contained a number of old scabs

from the lesions of the previous year. The new activity for that season at Oroville, however, was not noted until the middle of January, 1920. The results are partially indicated in table 11.

TABLE 11

NUMBER OF AFFECTED TWIGS PER TREE. SPRAYED OCTOBER 28, 1919

No. of trees	Treatment	1920		
		January 24	March 25	May 25
16	Bordeaux 5–5–50	1.5	5.4	14.8
12	Mercuric cyanid 1–500	1.7	7.0	12.7
19	Check	9.8	22.7	33.6*

* In this later count fifty-five trees were included as checks.

These figures give only a part of the results, since the twigs on the sprayed trees which did become affected were much less severely affected than twigs on the unsprayed trees. The sprayed trees stood out in striking contrast to the unsprayed trees, as being only slightly damaged. Almost no twigs on the sprayed trees were girdled and killed, while the unsprayed trees showed many twigs that had been killed back.

A very slight burning of leaves was noticed on the trees sprayed with mercuric cyanid, but none on the trees sprayed with Bordeaux Otherwise the general appearance of the trees was similar in the case of the two sprays. On eight trees of the Bordeaux plot and six of the mercuric cyanid plot, most of the dead twigs and old lesions of the previous year were dissected out just following the spraying. No appreciable difference could be detected as a result of this treatment. These trees, however, were surrounded by trees full of old lesions.

Another block of trees were sprayed with a three per cent Ortho lime-sulfur solution on October 29, 1919. In this block no difference could be detected between the sprayed and unsprayed trees. Other trees were sprayed with Bordeaux at later dates, some in January, 1920, and some in February. In the case of these later sprayings little difference could be detected between the sprayed trees and the unsprayed ones adjacent to them.

On December 17, 1919, experiments with soda Bordeaux (2 lbs. sal-soda, 2 lbs. copper sulfate, to 50 gallons of water) and Bordeaux were made in another orchard of navel orange trees about ten years of age. Records, which were taken on January 24 only, showed the following results (table 12):

TABLE 12

NUMBER OF AFFECTED TWIGS PER TREE. SPRAYED DECEMBER 17, 1919

No. of trees	Treatment	Jan. 24, 1920 (p. 69-70)
8	Bordeaux	10.5
9	Soda Bordeaux	10.7
10	Check	14.0

In the case of readings taken in March, 1920, no marked difference in amount of citrus blast was detectable upon general observation. Two blocks of nine trees each in a third orchard, which were sprayed with ammoniacal copper carbonate (3–6–50) and Ortho lime-sulfur (three per cent) respectively on December 16, 1919, showed no results which might help in controlling blast.

The second year, 1920–1921, a series of experiments were carried out as indicated in table 13. These were made on trees similar to those of table 11, which were by this time six years of age.

TABLE 13

NUMBER OF AFFECTED LESIONS PER TREE

No. of trees	Treatment	Number of lesions			Ratio of number of lesions on south side to that on north
		Dec. 16	Jan. 25	Feb. 16	
10	Bordeaux, Nov. 2	2.5	65	329	6
22	Bordeaux, Dec. 16	.....	139	395	3
11	Bordeaux, Nov. 2 and Dec. 16	2.8	71	233	5
11	Am. $CuCO_3$, Nov. 2	5.0	139	440	3
11	Bordeaux, Dec. 16, and Am. $CuCO_3$, Nov. 2	3.8	173	515	3.5
19	Check, east side of grove	18.2	262	658	3
13	Check, west side of grove	27.8	250	771	2.7

It will be noted that in this record the individual lesions, instead of the affected twigs, were counted. As in the case of the previous year, the figures indicate only partially the difference between the sprayed and unsprayed trees, as the lesions on the sprayed trees

appeared to average less than those on the unsprayed trees and therefore fewer branches were killed. The best results were obtained from the two plots sprayed on November 2. The plot which received a second spraying on December 16 appeared only slightly improved, and where the spraying was delayed till December 16 the benefit was still less. The results of the spraying with ammoniacal copper carbonate indicate that much less benefit was received in that case than from that with the Bordeaux.

The relative difference in number of lesions between the north and south sides of the trees, the reasons for which have already been discussed, was an interesting feature. On the unsprayed trees there were approximately one third as many lesions on the north as on the south side. On the trees sprayed with Bordeaux on November 2 there were one fifth and one sixth as many on the north as on the south side. The prevention, therefore, appeared to be relatively more effective on the north than on the south side. Possibly the driving rains from the south in the Oroville section would tend to lessen the relative efficiency on the south side. It is believed that these counts are fairly representative, as several tests in which counting was done from individual trees by different observers or repetitions which were made by the same observer showed that the variation in repeated counts was small.

During the third year, 1921–1922, several tests were made by growers. These varied as to time of application. The results point to the same conclusions regarding the importance of early fall spraying.

One grower sprayed part of an orchard of navel orange trees with Bordeaux (5–5–50) on November 1st to 3rd, 1921. About April 1, 1922, an examination of the trees sprayed showed almost no blast on them, while unsprayed adjacent trees were badly affected. A second block was sprayed November 28, 1921, but the results were not as satisfactory. On representative samples from these trees only one lesion to several hundred leaves was found on the twigs sprayed about November 1, while 10 to 25 per cent was the average number with twigs sprayed November 28, and about 50 per cent with twigs not sprayed.

Another grower sprayed with Bordeaux December 5, 1921, and although the prevention was not as effective as that which resulted in

the case above, the benefit appeared marked when comparison was made with contiguous unsprayed trees. Other sprayings made from December 10th to 15th showed differences detectable upon observation, but not sufficiently marked to be commercially successful.

The results of the experiments made in these three years all point to the conclusion that an early fall spraying (about November 1) is more efficient for prevention of citrus blast than later ones, even though the activity of the blast apparently seldom begins before January. The reason for this fact is not known.

RECOMMENDATIONS

1. Spray with Bordeaux mixture as early as November 1. A second spraying on or before December 15 may be helpful, but the first alone is much more effective than the second alone. The spraying should be thorough in order to cover the entire surfaces of the twigs and leaves. It is therefore suggested that a spreader such as casein be added.

2. When it is possible, grow a tree with a bushy, compact type of growth, having a minimum liability to injury during severe wind storms.

3. In the case of new planting of citrus provide some means of protection against injury caused by the south winds.

These suggestions are of little importance for conditions in southern California, since the disease is a minor one there, usually manifesting itself only in black pit of the fruit which is seldom of sufficient importance to demand a remedy. Spraying trees with Bordeaux would be detrimental in places where fumigation with cyanid is practiced. With regard to protection against injuries the same principles apply to all sections.

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PLATE 1

Citrus blast lesions on leaves and twigs of Navel orange.

Fig. A. Active lesions starting in February from infection at a tear caused by the wind on the wing of the petiole. Points of infection indicated by the arrows.

Figs. B and C. Inactive lesions of the previous year on live twigs, showing by the size and condition of scabs some of the different degrees of severity in the development of the disease. B shows an average condition at Oroville while C shows a very severe development.

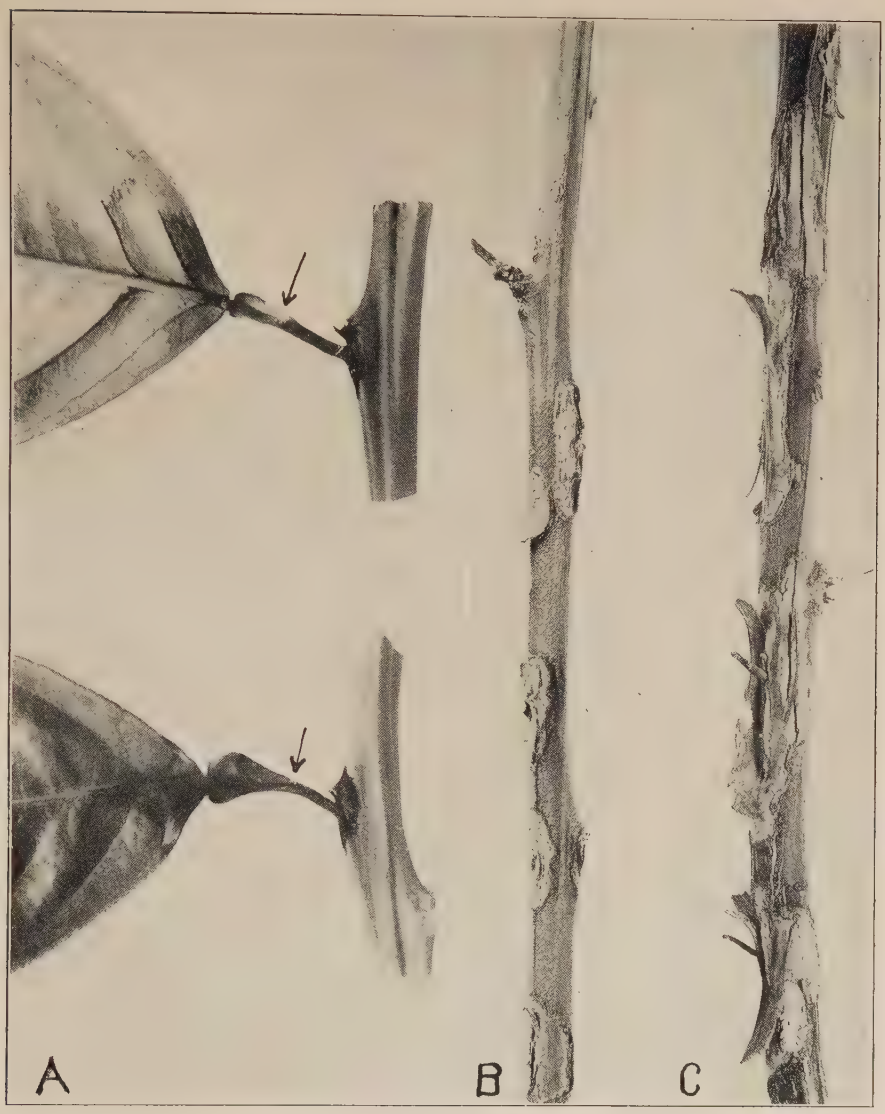
A
B
C

PLATE 2

Showing small lesions surrounding attached petioles. These have attained their maximum development at the end of the active period. Near the center is a typical example of the many dead dry leaves which frequently remain firmly attached through the subsequent summer and fall.

PLATE 3

Fig. A. Blast on stem and leaf of Navel orange. The arrow points to the tear in the wing of the petiole where infection probably started and spread in both directions to the leaf blade and to the twig which is being killed back. A surface stain or scorch has also spread out over one portion of the leaf blade.

Fig. B. Blast on the leaf blade and black pit next to it on the fruit of the lemon.

A
B

PLATE 4

Black pit spots developing from natural infections in slight injuries to fruit, Oroville, California, February, 1921. *Pseudomonas citriputeale* was isolated from some of these lesions in each species. A, Navel ornage. B, Grapefruit. C, Three lemons. On several of the spots slight scratches which opened the way for infection are shown.

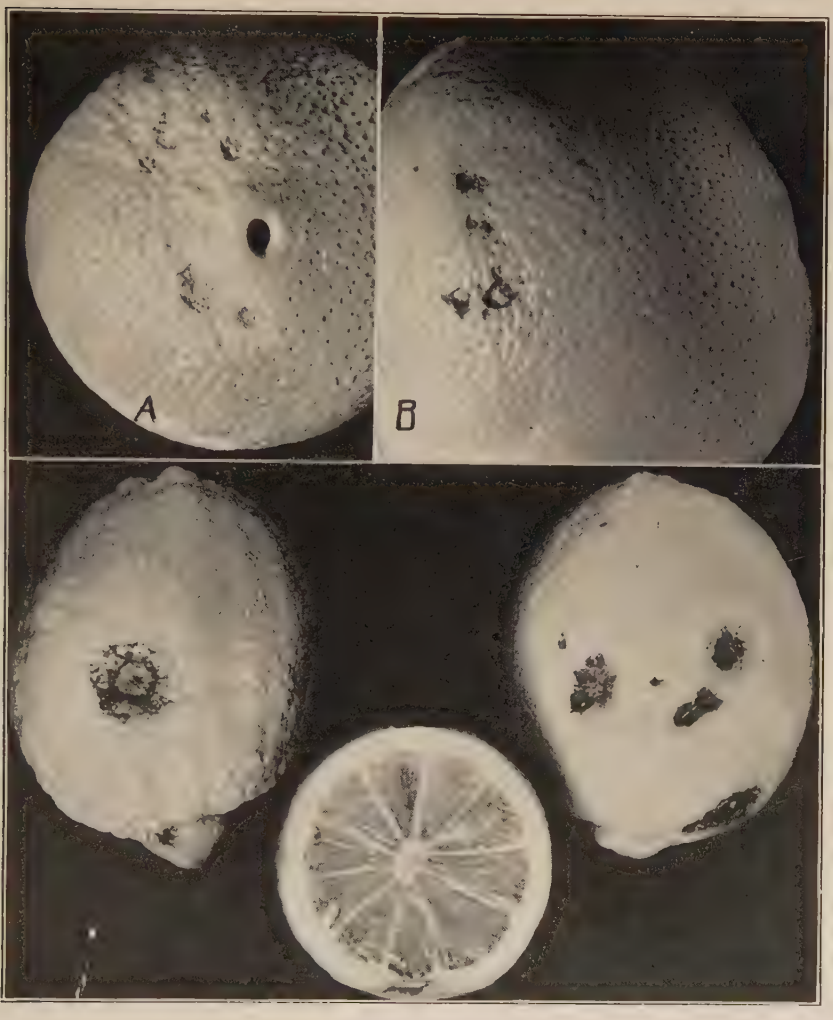
A
B

PLATE 5

963 shows black pit spots on a lemon grown near Alta Loma in southern California. The spots shown on 1001 were produced by inoculating with a culture isolated from a citrus blast lesion on a Navel orange twig grown near Oroville in northern California.

963
1001

PLATE 6

Three citrus blast lesions on orange leaves. Two upper lesions produced by inoculations with culture of *Pseudomonas citriputeale* isolated from black pit spots on lemons grown in southern California, and lower by culture isolated from citrus blast lesion on orange twig grown in northern California.

K
GRAB
1 Cult 967 (P.F.B.P.
May 28
Orange
2. Cult 7a
May 28
Orange